

REMARKS

The above amendments to the specification and claims have been made to be in compliance with CFR §1.821-1.825. Accordingly, Applicants believe no new matter is added by these amendments.

CONCLUSION

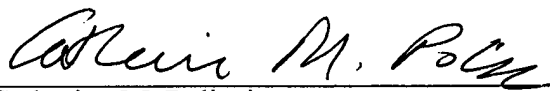
Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made".

In the unlikely event that the fee transmittal is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 220002060310. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please substitute the following for the paragraph beginning on page 21, line 13 and ending on page 22, line 9

cDNAs were subcloned into the mammalian expression vector pBJ1. For receptor cleavage studies Cos 7 cells were transfected using DEAE-dextran and thrombin-mediated loss of M1 antibody (Kodak) binding to the FLAG epitope of the cell surface using a procedure described by Ishii et al. (Ishii, K. et al. (1993) *supra*). Over 95% of M1 antibody binding was transfection-dependent in this system. Cells were incubated for 5 min. at 37°C in the presence (open columns) or absence (closed columns) of 20nM thrombin (Fig. 6). For biochemical identification of the cleavage site, cleavage of soluble PAR3 amino terminal exodomain by thrombin was assayed as follows. A recombinant PAR3 soluble exodomain was prepared in which the amino terminal exodomain residues 21-94 were sandwiched between a translational start and hexahistidine tag (i.e. MG- [PAR3 21-94] -VEHHHHHH (SEQ ID NO:29); where VEHHHHHH is SEQ ID NO:18). The recombinant protein was expressed as a soluble polypeptide in *E. coli*, purified, and analyzed before and after thrombin cleavage as previously described for the analogous region of PAR1 (Ishii, K. (1995) J. Biol. Chem. 270:16435-16440). Recombinant soluble amino terminal exodomain was cleaved in solution with 50nM thrombin for 1h at 37°C, then analyzed by SDS-PAGE. Even prolonged incubation with a high concentration of thrombin yielded only one detectable cleavage event indicting that only one thrombin cleavage site exists in the PAR3 exodomain. Amino acid sequencing of the cleavage products revealed only a single new amino terminus with the sequence TFRG [(see Fig.1b)](see Fig. 3). Thus, thrombin recognizes and cleaves PAR3 in the amino terminal exodomain between amino acids K38 and T39 with high specificity.